

Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

Studies on Dietary Requirements for Lactation.

IV.—On the Nature of Factor L, a Specific Dietary Factor for Lactation.*

By Waro NAKAHARA, Fumito INUKAI, and Saburo UGAMI.

(Received December 3, 1935.)

INTRODUCTION.

The idea that there may exist a specific dietary factor for lactation has recently been suggested in literature, but the failure definitely to discriminate this hypothetical factor from vitamin B complex rendered difficult the establishment of its actual existence (Mapson⁽¹⁾ Smith and Seegers⁽²⁾).

In May, 1934, we⁽³⁾ first succeeded in demonstrating that the lactation promoting substance in liver extract is not adsorbed by acid earth under the condition in which vitamin B is completely adsorbed. The result made it quite clear that the lactation factor is entirely different from vitamin B complex, and formed the first firm ground for the existence of a specific dietary factor for lactation. For this unidentified substance we proposed the descriptive designation of "Factor L". The fact that sufficiently purified vitamin B complex, even in a very large amount, cannot support lactation, and that therefore another factor is necessary for the latter process has more recently been shown by Feaster and Nelson.⁽⁴⁾

It may be worth while to point out here, in order to avoid possible confusion, that by "Factor L" we mean an essential dietary factor, without which a total failure of lactation results. Increase by dietary means of the level of milk secretion already established is an entirely different question, since all the work of this nature depends on basal diets containing factor L. The recent experiments by Daggs and Tombouljian,⁽⁵⁾ who showed cystine

* Previously published in Scientific Papers of the Institute of Physical and Chemical Research, Vol. 28, December, 1935.

or glutathione to be a mammary stimulant come under this category, and these must be regarded as having no direct bearing on the question of factor L.

For the past year we have been engaged in studies on the chemical nature of factor L, and so far, in addition to the fact that the unidentified substance in question is not adsorbed by acid earth, we found that it is precipitated from the non-adsorbable fraction by barium hydroxide and methanol, and from the decomposed barium precipitate by phosphotungstic acid. The final phosphotungstic precipitate we obtained proved to be active as factor L in daily doses of less than 50 mg per rat.

The details of our experiments covering these new results will be given in the following lines.

METHOD OF LACTATION TEST.

The experience of the past few years in the investigation of factor L taught us the main facts as to the nature of experimental procedures involved, and we have now been able to greatly simplify the method of lactation test described in our first paper.⁽⁶⁾ A brief account of our improved method may not be superfluous at this time.

Basal diet :— The basal diet used was the same as in our previous experiments, with the following composition :

Polished rice powder	75 g
Fish protein	10 g
Butter	10 g
McCollum's salt mixture	5 g
Dried yeast (Oriental brand)*	5 g

This synthetic diet was tested and proved to be entirely adequate for rats for all purposes, except for lactation, and in spite of the excellent growth of the animal, normal pregnancy and parturition, lactation fails on this diet in practically one hundred per cent of the cases.

We learned empirically that female rats, previously maintained on the

* This brand of yeast seems exceptionally poor in factor L. We found that lactation was not markedly improved by increasing the yeast content of the above diet to 20 g. as shown in the following result of lactation test :—

Female No.	Number of young born	Number of young weaned	Female No.	Number of young born	Number of young weaned
1	11	0	7	8	0
2	6	6	8	8	0
3	10	0	9	11	0
4	6	0	10	8	3
5	7	0	11	10	3
6	9	0			
			Total	94	12 (12.76%)

usual natural food, become sufficiently factor L. deficient for experimental purpose in two weeks after being transferred to the above basal diet. Pregnant females maintained on this diet for just two weeks preceding parturition always showed a total failure of lactation, the young surviving only for a few days after birth.† With this knowledge we planned a standard method for lactation test as follows :-

Lactation test :- A number of healthy virgin female albino rats, raised on the usual natural food of grains and green vegetables until they attained the body weight of 130~150 g, were maintained on the factor L. deficient basal diet described above.

After one week, large healthy male rats with body weights of over 200 g, previously fed on natural food, were paired off with the females, and cohabitation was allowed for one week, after which all the males were removed. This cohabitation period of one week was adopted more for convenience than for necessity, since most of the females were fertilized during the first four or five days of cohabitation.

Upon the removal of the males, i.e., after 2 weeks of the preliminary feeding on the basal diet, the material to be tested for its lactation promoting action was added to the diet, and, on this supplemented diet, females were allowed to go through the periods of gestation and parturition, and lactation, if capable. In this way, females were fed on supplemented diet usually for 16~19 days before parturition, in no case for less than two weeks or longer than three weeks. Any female showing no definite sign of pregnancy by the end of two weeks after removing the male was discarded as non pregnant. Usually we found two or three such females out of every ten.

† Our data bearing on the question of the time required to produce factor L. deficiency consist of observations on 11 rats which were already pregnant when they were first placed on our L-deficient diet. The number of days on this diet before parturition and the ratio of the successful lactation for these 11 rats were as follows :-

Female No.	L deficient feeding before parturition	Number of y ung born	Number of young weaned
1	9 days	8	3
2	11 "	5	0
3	12 "	8	0
4	" "	10	4
5	" "	10	3
Total	9~12 "	41	10 (24.39%)
6	14 "	7	0
7	" "	10	0
8	" "	8	0
9	18 "	9	0
10	19 "	3	0
11	" "	5	0
Total	14~19 "	42	0 (0.00%)

As soon as approaching parturition was detected, each female was placed in a separate cage with a suitable amount of straw.

Success or failure of lactation was determinable usually in two or three days after parturition, and the females suckling the young were continued on supplemented diet for three weeks after parturition, at the end of which period the young were weaned and their body weight recorded. The true lactation period of the rat is 16 or 17 days, since at about this time the young open their eyes and start nibbling at the mother's food. For safety, however, we allowed full three weeks before weaning. All through the lactation test care was taken to avoid all unnecessary disturbance of the animals.

The ratio of the number of young weaned against the number born was taken as the index of the lactation. The weight of the young at weaning was regarded as only of incidental interest, since we did not reduce the number of the young to be suckled to any standard, thus rendering the weaning weight of the young practically meaningless. The nature of our test being such that the ratio of young weaned against young born constituted the all-important criterion.

PRECIPITATION BY BARIUM HYDROXIDE.

We have already proved that factor L, existing in liver extract is not adsorbed by acid earth. Our first attempt in the present investigation was to determine whether or not this unidentified substance is precipitated from the non-adsorbable fraction by barium hydroxide.

Fresh beef liver was ground fine in a mortar and was thoroughly extracted with 40% alcohol acidified with a small amount of 25% sulphuric acid to pH 3~4, using 5 litres of the solvent per 1 kg of liver. White acid earth was added to this extract at the ratio of 30 g per 1 litre, and the mixture was maintained at 30°C for 20 minutes with occasional stirring, after which the acid earth was filtered off.

The filtrate, which our previous experiments showed to be the active fraction, was neutralized with barium hydroxide, thus removing excess sulphuric acid, and was concentrated under reduced pressure and at a low temperature so as to reduce the volume of extract from 1 kg of liver to less than 150 cc. To this concentrated extract was added finely pulverized barium hydroxide in excess, and the mixture was frequently agitated until it generated a strong ammonia odor. The mixture was then poured into about ten times its volume of absolute methanol with vigorous stirring, and the stirring continued for 2~3 hours more with a stirring machine. After this treatment the mixture was allowed to stand for 24 hours, and then the precipitate was separated from the filtrate.

The precipitate, the bulk of which apparently consisted of barium salt

of glutamic acid, was dissolved in water and insoluble matter (glycogen fraction) was removed. Barium was then precipitated with H_2SO_4 and removed, and finally the solution was neutralized with NaOH.

The filtrate, which contains most of the organic bases in liver extract, was first freed of methanol, barium was removed with H_2SO_4 , and the remaining base constituents were neutralized with HCl. The solution was then concentrated into a suitable volume.

Both precipitate and filtrate were added to every 100 g of basal diet in amounts corresponding to the yield from 100 g of fresh liver.

The result of lactation tests carried out in accordance with our standard method was as follows (Table I):

TABLE I—*Effect of barium precipitate and filtrate on lactation.*

A. Precipitate.

Female No.	Number of young born	Number of young weaned	Average body weight of young at weaning
1	3 (All died in 4 days)	0	—
2	12 (None died)	12	23.66 g
3	10 (1 died in 5 days)	9	25.66 g
4	7 (2 " 6 ")	6	26.00 g
5	8 (2 " 4 ")	6	39.66 g
6	5 (All died in 4 days)	0	—
7	11 (" 4 ")	0	—
8	8 (" 3 ")	0	—
Total	64	33 (51.56%)	

B. Filtrate.

1	11 (All died in 3 days)	0	—
2	7 (" 2 ")	0	—
3	9 (" 2 ")	0	—
4	6 (None died)	6	26.00 g
5	12 (All died in 3 days)	0	—
6	8 (" 3 ")	0	—
7	5 (" 1 ")	0	—
8	9 (" 2 ")	0	—
9	5 (None died)	5	34.80 g
Total	72	11 (15.27%)	

The above results indicate that factor L is precipitated by barium hydroxide and methanol.

PRECIPITATION BY PHOSPHOTUNGSTIC ACID.

The barium precipitate, which the preceding experiment showed to be the active fraction, was first freed of barium with H_2SO_4 , and after being concentrated to a suitable volume, and adding H_2SO_4 in the proportion of 3%, was precipitated with phosphotungstic acid.

The precipitate was decomposed with barium hydroxide according to the usual method, barium was removed with H_2SO_4 , excess H_2SO_4 neutralized with NaOH , and the solution was suitably concentrated. The yield of this fraction from 100 g of fresh liver was 176 mg, this amount including the extraneous H_2SO_4 and NaOH which were added as just stated.

The filtrate was treated with barium hydroxide to remove H_2SO_4 as well as $\text{WO}_3 \cdot 2\text{H}_3\text{PO}_4$, and excess barium was removed with H_2SO_4 . The resultant solution was strongly acid and was therefore neutralized with NaOH . It was then concentrated into an appropriate volume.

Both precipitate and filtrate prepared as above were added to 100 g of basal diet in amounts corresponding to the yield from 100 g of fresh liver. In the case of the precipitate this amount was about 170 mg. Since the daily amount of diet offered to a rat was about 30 g on an average, the amount of the precipitate may be estimated at 51 mg per rat per day. It must be added, however, that the rats by no means completely consumed their daily portion of diet.

The result of lactation tests was as follows (Table II):

TABLE II—*Effect of phosphotungstic acid precipitate and filtrate on lactation.*

A. Precipitate.

Female No.	Number of young born	Number of young weaned	Body weight of young at weaning
1	7 (None died)	7	31.85 g
2	10 (All died in 3 days)	0	—
3	9 (None died)	9	27.33 g
4	7 (All died in 5 days)	0	—
5	8 (None died)	8	31.00 g
Total	41	24 (58.56%)	

B. Filtrate.

1	8 (All died in 3 days)	0	—
2	8 (" 4 ")	0	—
3	10 (6 died in 9 days)	4	26.75 g
4	8 (All died in 4 days)	0	—
5	9 (" 3 ")	0	—
6	8 (1 died in 9 days)	7	24.28 g
7	5 (All died in 3 days)	0	—
Total	56	11 (19.82%)	

The above result shows that the phosphotungstic precipitate carries most of the lactation factor. Glutamic acid which made up the bulk of the active barium precipitate is not precipitated by phosphotungstic acid, and is therefore to be eliminated from consideration.

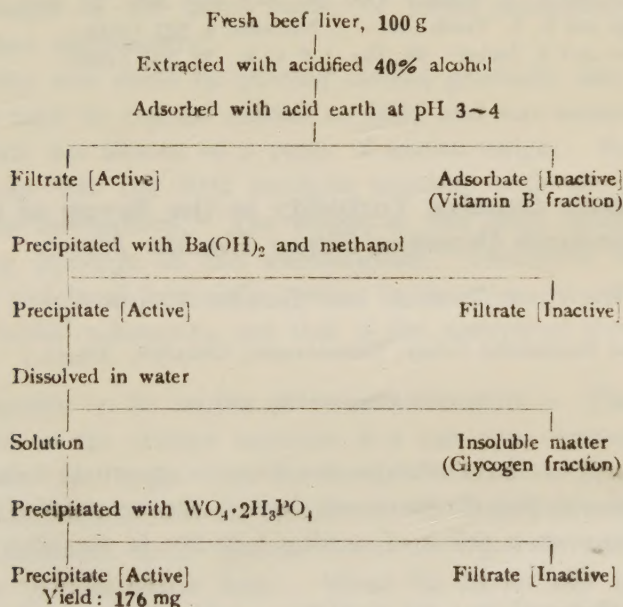
DISCUSSION AND SUMMARY.

One point in the results of these lactation tests that appears somewhat

strange is the fact that, even under the best conditions the ratio of successful lactation scarcely reached 60 per cent. Why this should be we are not prepared to answer. It is possible that the diet is still imperfect either qualitatively or quantitatively, but it would seem more likely that some physiological mechanisms outside of the dietary control may be involved in the case. Whatever be the final explanation of this phenomenon, however, the contrast between the practically total failure of lactation on the otherwise complete diet and over 50 per cent of successful lactation due to the supplement of a small amount of an unidentified substance must be regarded as sufficiently striking.

The steps so far employed by us in attempt to purify factor L may be schematically summarized in Table III.

TABLE III—*Preparation of crude factor L.*



The fact that the substance which is active as factor L is not adsorbed by acid earth but is precipitated by barium hydroxide may indicate that the substance has a character of acids, while the precipitability by phosphotungstic acid may conceivably be taken to mean that here we may be dealing with a nitrogenous substance. However, speculations as to the exact chemical nature of factor L are useless without further purification.

It is worthy of special attention that the crude phosphotungstic precipitate we obtained was effective in the daily amount of less than 50 mg per rat. That this amount of the crude preparation proved to be capable of preventing the failure of lactation indicates that, upon further purification, the unidentified

substance (factor I.) will be found to be active in very small doses. If the total failure of lactation on the otherwise complete synthetic diet can be prevented by a minute amount of the substance in question, it would seem that the latter may reasonably be classed under the category of vitamins.

In conclusion, we take the pleasure of expressing our sincere appreciation to Professor U. Suzuki, M. I. A., for his cordial encouragement and valuable criticisms.

LITERATURE

- (1) L. W. Mapson: *Biochem. J.*, **26**, 970 (1932).
- (2) H. G. Smith and W. H. Seegers: *J. Nutrition*, **7**, 209 (1934).
- (3) W. Nakahara, F. Inukai and S. Kato: *Proc. Imp. Acad.*, **10**, 268 (1934); *Sc. Pap. I. P. C. R.*, **24**, 155 (1934).
- (4) J. F. Feaster and V. E. Nelson: *Proc. Soc. Exp. Biol. Med.*, **32**, 1456 (1935).
- (5) R. G. Daggs and R. L. Tomboulion: *J. Nutrition*, **5**, 581 (1935).
- (6) W. Nakahara and F. Inukai: *Sc. Pap. I. P. C. R.*, **22**, 301 (1932).

On a Substance Causing Turbidity in the Syrup of Canned Mandarin Oranges (*Citrus Unshiu Marc*).

By Yasuo IWASAKI and Tatsuya SUGIMOTO.

(Chiba Horticultural College, Matsudo-machi, Chiba-ken, Nippon.)

(Received December 21, 1935.)

The canning of mandarin oranges has recently grown to a large industry in Nippon, and occupies the foremost place in the exportation list of this kind. It has, however, a defect of causing turbidity in the syrup after it is manufactured.

The authors observed that the turbidity consisted of two substances, one is amorphous and the other white crystals of very fine needles. So the authors studied the crystalline substance particularly and isolated hesperidin by the following method.

Isolation of the crystalline substance:- The syrup of canned mandarin oranges was centrifuged, and the sediment in the tubes was sacked on a filter paper and dried up in a desiccator. This dried substance was dissolved with 50% alcohol on the boiling water bath. The yellowish solution was filtered in order to remove some insoluble matter. After standing or evaporating a little, light yellow needles were separated from the filtrate. These were redissolved with 50% alcohol containing a few drops of HCl on the boiling water bath. In the filtrate, the crystal reappeared beautifully, it

gives reactions of hesperidin. The melting point is $250 \sim 251^{\circ}\text{C}$ (uncorr.).

Analyses of hesperidin :

0.1732 g Subst.,	0.3482 g CO_2 ,	0.0927 g H_2O .
0.1715 " "	0.3435 " "	0.0923 " "

	C %	H %
found	54.83	5.95
	54.62	5.97
calc. for $\text{C}_{50}\text{H}_{60}\text{O}_{27}$	54.95	5.50
" " $\text{C}_{28}\text{H}_{35}\text{O}_{15}$	54.09	5.57

Hesperidin content in the turbid syrup :— After removing the fine pulps of the oranges from 100 cc of the syrup passed through a fine sieve, the turbid substances were filtered upon a dried filter paper of known weight. The total weight of the dried matter of turbid substances was 0.0220 g (average of two experiments).

A solution was made by pouring alcohol gradually into another 100 cc of the syrup until its content reaches to 50% and was warmed on the water bath and while hot filtered on a paper of known weight. By this treatment hesperidin goes into the 50% alcoholic solution, while the amorphous substance remains undissolved. The weight of this dried matter on the paper was 0.0094 g (average of two experiments). Calculated from the above results, the weight of hesperidin content in the syrup was 0.0126 g = 57.3% of the total turbid substances, and that of the amorphous crystal was 0.0094 g = 42.7%.

White speckles on the surface of the skinned segments :— The white speckles on the surface of the skinned segments in a can were observed by the naked eyes to a size not larger than the head of needles or pins and were seen through a microscope to be white needles or tuft-shaped or amorphous crystals. They were collected by a pincette from the segments and dissolved with methanol on the hot water bath. When the filtrate was concentrated, the white crystal was produced. It was purified by the method described above. The melting point was $250 \sim 251^{\circ}\text{C}$ (uncorr.). The white speckles on the segments, thus prepared, showed the same crystal form and the same melting point with those of pure hesperidin and properties and reactions of the both were identical in every respect.

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

The Effect of Yeast upon Malnutrition of Rats Caused by High-sucrose Ration (Preliminary report). (pp. 1~10): By Hisasi ARIYAMA. (Agr. Chemical Laboratory of Tokio Imperial University, Tokyo.)

In the previous paper, the author pointed out that regardless of the sufficient supply of all the known vitamin, the albino rats could hardly thrive on the high-sucrose diet which contained the sugar to the extent of 60% or more while the animals on the same diet, supplemented with dry brewery yeast (0.5 g yeast per diem), showed nearly normal growth. In the present work, the author made some investigations into the nature of the active substance contained in yeast and obtained the following results:

(1) The active substance was readily extracted by dilute alcohol but not easily extracted by alcohol of higher concentration.

(2) The active substance was not precipitated by lead-acetate and was well adsorbed on acid clay.

(3) When the alcoholic extract of yeast was submitted to autoclaving for 4 hours at 120° and pH 5.6 (natural pH of the yeast extract), about one-half of the active substance survived, while at pH 8.0, was practically all destroyed.

(4) Alcoholic extracts of ox-liver and green leaves, i.e. lettuce and spinach, also exhibited a beneficial effect upon the rats fed on the sugar diet but their potency was nearly half of that of the yeast extract.

(5) Increasing supply of vitamin B₁ (B₁-crystal) or B₂ (Narayanan & Drummond's B₂-concentrate or egg-white) had scarcely shown any favorable effect upon the animals. Also the administration of vitamin B₁-concentrate, prepared by the method of Barnes, O'Brien and Reader, showed no sign of positive effect.

From the experimental data described above, the author came to the conclusion that, to maintain the normal metabolic process of animals fed on diets of high sugar content, some special dietary factor (or factors), which is different from hitherto known water soluble vitamins, is need.

Studies on "Kaoliang" as the Source of Starch-making,

Milling, and "Ame"-manufacture. I.—The Distribution of Constituents in "Kaoliang"-Grains. (pp. 11~18): By R. YAMAMOTO. (Central Laboratory, South Manchurian Railway Co.)

The composition of kaoliang (*Andropogon Sorghum*, Brot) and its various parts together with the distribution of constituents in germ, bran and endosperm are given.

Studies on "Kaoliang" as the Source of Starch-making, Milling, and "Ame"-manufacture. II.—The alcohol soluble proteins of glutenous and common Kaoliang. (pp. 19~25): By Mamoru NINOMIYA and Ryôji YAMAMOTO. (Central Laboratory, South Manchurian Railway Co.)

1. Some properties of alcohol soluble proteins, prepared from the glutenous and common Kaoliang, were compared. There could not be found any significant differences between them.

2. The iso-electric point of the alcohol soluble proteins of Kaoliang is less acidic than that of Kafirin reported by Hoffmann and Gortner.

3. The digestibility of the alcohol soluble proteins of Kaoliang is somewhat higher than that of zein, but lower than that of oryzenin.

Studies on Germ and Outer Seed Coat (Bran) of Kaoliang Grains. (IV).—A wax and hydrocarbon in bran. (pp. 26~28): By Ryôji YAMAMOTO and Mamoru NINOMIYA. (Central Laboratory, South Manchurian Railway Co.)

In the previous paper (*J. Agr. Chem. Soc. Japan*, 10, 248, 1934), the authors stated that when the ether extractives obtained from the outer seed coat (bran) of Kaoliang Grains were treated with petroleum ether, there remained a insoluble resinous or waxy fraction, corresponding to ca. 5% of the ether extractives.

In the present research, the petroleumether-insoluble fraction was investigated and a hydrocarbon having somewhat higher melting point ($78\sim79^\circ$); reflex index (n_D^{25}) 1.4368, and a wax having melting point $79\sim80^\circ$, saponification value 69.3 were obtained.

As the constituents of the wax, arachidic acid, cerylalcohol and another alcohol which melting point $83\sim84^\circ$ and that of its acetylated product $68\sim69^\circ$, were proved. From the yield of arachidic acid and the quantity of cerylalcohol corresponding to arachidic acid, it is presumed that the wax is composed of ca. 19% of arachidic acid and 81% of free alcohol. By the Liebermann's starch reaction upon the petroleum ether-insoluble fraction, the author, also presumed the presence of a certain resinic acid.

Studies on the Germ and Seed Coat of Kaoliang. (V).—The Phosphatides of the Kaoliang Germ. (pp. 29~32): by Ryôji YAMAMOTO and

Mamoru NINOMIYA. (Central Laboratory, South Manchurian Railway Co.)

1. Before fractionation of a preparation of the phosphatides of Kaoliang germ, analyses for the phosphor distribution were conducted.

2. The deductions made from this analyses were confirmed by a detailed investigation of 3.3 g of the phosphatide fractions prepared from 16 kg. of Kaoliang germ.

3. This fractionation showed the presence of kephalin and lecithin. These substances occur in the approximate proportions of 3:1, when referred to the yield of respective fraction.

Untersuchungen von Essigbakterien aus Formosa (VII). (S. 33~55): Von Syoosuke TANAKA. (Department of Industry, Government Research Institute, Formosa, Japan.)

On the Soil formation in North-Kyushu. (pp. 56~61): R. KAWASHIMA. (Agr. Chemical Laboratory, Kyushu Imperial University.)

1. The soils formed from sedimentary and schistose rocks in North-Kyushu were chemically analysed.

2. The climatic condition of related districts is warm and wet as represented in the following table.

Locality	Mean temp, (°C)	Mean precipitation (mm)	Relative humidity	Meyer's NS-Quotient
Fukuoka	14.9	1586.7	77.9	571
Simonoseki	15.2	1638.5	75.3	517

3. Most soils are extremely poor in bases and strongly acidic.

4. The reaction and exchangeable CaO of these soils are as follows;

Soil	Geological formation	pH		Exchange acidity ($y_1 \times 3$)	Exchangeable CaO (%)	% of exchange- able CaO for HCl-soluble CaO
		H ₂ O	KCl			
Fuku. 1	Tertiary shale	4.29	3.80	182.4	Not determined	—
" 2	"	4.87	3.74	442.9		—
Hiko. 1	Cretaceous shale	4.89	3.88	69.2	0.075	51.7
" 2	"	4.62	3.94	58.0	0.068	54.0
Kawa. 1	Paleozoic slate	3.77	3.54	73.8	0.014	17.7
Ka. 1	"	4.47	3.62	58.0	0.024	24.5
Yobu. 1	Paleozoic limestone	3.63	3.39	187.2	0.004	3.7
" 2	"	6.11	6.00	basic	0.325	27.5
Kawa. 2	Chlorite schist	4.40	3.44	119.8	0.006	5.0
Wakasugi	Talc schist	4.14	3.53	88.8	0.010	1.9

5. Clay fractions below 0.001 mm in diameter were separated by settling method and analysed by using both Hardy's Tri-acid mixture and hot conc. HCl. From the analytical results $\text{SiO}_2/\text{Al}_2\text{O}_3$ and $\text{SiO}_2/\text{R}_2\text{O}_3$ are given.

Soil	Geological formation	By Tri-acid		By HCl	
		$\text{SiO}_2/\text{Al}_2\text{O}_3$	$\text{SiO}_2/\text{R}_2\text{O}_3$	$\text{SiO}_2/\text{Al}_2\text{O}_3$	$\text{SiO}_2/\text{R}_2\text{O}_3$ *
Fuku. 1	Tertiary shale	1.99	1.64	2.24	1.66
" . 2	"	2.60	2.12	3.28	2.53
Hiko. 1	Cretaceous shale	2.59	1.59	4.17	1.81
" . 2	"	2.27	1.61	3.25	1.75
Kawa. 1	Paleozoic slate	1.94	1.31	2.20	1.27
Ka. 1	"	2.40	1.79	2.80	1.78
Yobu. 1	Paleozoic limestone	1.89	1.25	1.84	1.04
" . 4	"	2.22	1.73	4.04	2.59
Kawa. 2	Chlorite schist	1.93	1.35	1.46	0.95
Wakasugi	Talc schist	2.41	1.63	2.49	1.58

6. Considering the above tables, the author may conclude that the soil formation in North-Kyushu, in spite of the difference of their geological origin, is allit-siallitic.

The Changes of Oxidation-reduction Potentials of Water-logged Soils. (pp. 62~73): By Kisaburo SHIBUYA, Hideaki SAEKI and Kenhan RYU. (Chemical Laboratory for Soils and Fertilizers, Taihoku Imperial University.)

It is dealt with, in this paper, the changes of oxidation-reduction potentials of soils which were in water-logging condition for 100 days. They were constantly kept at 30°C in a large thermostat. The soil samples were taken 6 kinds of different types, viz. lateritic-, alkali-, humus-, "kantenden"-soil and soils derived from clayslate and sandstone. The apparatus and the method applied to this investigation mostly followed after those which were proposed by Brown (18). The result of this investigation is summarized as it follows:

A) Oxidation-reduction potentials of air dried soils.

1. By measuring the potentials of soils, it is known that these are in order of humus soil > clay-slate soils > sandstone-shale soil > "kantenden" soil > lateritic soil > alkali soil (Table 1).

2. A relationship between oxidation-reduction potentials and pH values of the same soils changes by an inverse linear-relation. The soils of the higher pH value possess the weaker oxidizing power. Oxidation-reduction

potentials of the soils are at lower position or reducing side of the quinhydrone line (Fig. 1).

3. There should be some intimate relation between amount of ferrous oxide and oxidation-reduction potential of a soil but no definite proportionality, since various other factors affected on the potential (Table 2).

B) Oxidation-reduction potentials of water-logged soils.

1. The soils, with no exception, depress the oxidation-reduction potentials by submerging in water for a certain period, increasing their reducing power (Fig. 2).

2. The rapid depression of the potentials takes place within the first one week and comes to the minimum point at about fifth week. Change of the potentials, thereafter, is in almost constant or it comes up to a little extent.

3. The minimum oxidation-reduction potentials of the soils, except alkali soil, ultimately show negative values. It is doubtlessly suggested that hydrogen pressure in the phase is stronger than the oxygen pressure.

4. The soils which noticeably decrease the oxidation-reduction potentials in their water logged condition, contain a large amount of humus or ferrous compounds.

5. Quantity of ferrous compounds in soils increases by water-logging.

6. The oxidation-reduction potentials of soils in a water-logged condition change by a close connection with pH values of the soils.

7. Oxidation-reduction potentials of soils are affected by pH of the soils, as they are in theoretical relation $Eh = -0.06 \text{ pH}$ (30°C). The measured potentials, therefore, are corrected by potentials calculated from that equation, eliminating influences of pH. It is suggested, in consequence, that changes of the potentials of soils in water-logging condition are not only influenced by change of their pH, but by other factors.

On the Phytase and the Glycerophosphatase of Rhizopus. (pp. 74~81): By Kohei MIYAGAWA, (Agr. Chemical Laboratory, Hokkaido Imperial University.)

On the Nutritive Value of Biscuit. (pp. 82~86): By Rinjiro SASAKI and Norihide ANDO. (Agricultural Chemical Laboratory, Tokyo Imperial University.)

On the Enzymes in the Yellow Tobacco. (I). (pp. 87~95): By T. NITO and E. KITAMURA. (The Mito Experiment Station, Government Monopoly Bureau, Japan.)

This investigation has been done to study the fate of the enzymes in the yellow tobacco by the following division:—

1. Fresh leaves.
2. Flue-cured leaves.
3. Unfermented leaves.
4. Fermented leaves.

The leaves cut in slice, were extracted with some water and the filtrates free from the residue were used for the samples.

TABLE

Division	Fresh leaves	Flue-cured	Unfermented (redried)	Fermented		
				No. 1	No. 2	No. 3
pH	5.3	6.1	6.5	6.2	5.7	5.6
Diastase	+	+	+	+	+	very weak
Invertase	+	+	+	+	+	+
Maltase	—	—	—	—	—	—
Lipase	+	+	weak	—	—	—
Emulsin	+	+	very weak	—	—	—
Pepsin	—	—	—	—	—	—
Trypsin	+	—	—	—	—	—
Erepsin	+	+	+	+	—	—
Desamidase	—	—	—	—	—	—
Oxydase	+	+	+	weak	—	—
Peroxydase	+	weak	very weak	—	—	—
Catalase	+	+	—	—	—	—

- N.B. 1. The samples of No. 1 and 2 were fermented for one year.
 2. The sample of No. 3 was fermented for four years.
 3. The samples of No. 2 and 3 were produced in U.S.A.

On the Optical Properties of the Fermentation Lactic Acids.

Part II.—The manner of lactic acid bacteria towards inactive and active lactic acids added to the Fermentation liquids. (pp. 96~100): By Hideo KATAGIRI and Kakuo KITAHARA. (Laboratory for Physiology of Fermentation, Faculty of Agriculture, Kyoto Imperial University.)

In the present paper, lactic acid fermentations were carried out in the liquids containing nearly 2 g of a known form of lactic acid and a larger amount (about 10 g) of glucose, in which any selective decomposition of the lactic acid previously added, was not observed.

In the presence of inactive lactic acid, no modification of the form of lactic acid produced from glucose was observed by any of the lactic acid bacteria: *l*-former (*Leuconostoc mesenteroides* var. Sake), *d*-formers (*Lactobacillus* Sake and *Bacillus lactis brevis* Mongoricus) or *dl*-formers (*Lactobacillus*

Sake, *Lactobacillus plantarum* sp. and *Lactobacillus pentoaceticus*).

As the other *d*-formers (see Part I), any modification of the form of lactic acid produced by *B. lactis brevis* Mongoricus was never detected even when *d*- or *l*-lactic acid was added to the fermentation liquids.

However, *Streptococcus lactis bulgaricus* (*dl* + *d*-former) produced more *d*-lactic acid, in the presence of inactive lactic acid, so that the form of the fermentation lactic acid was identical to its own products.

The special nature (see Part I) of *dl*-formers in modifying the form of lactic acid produced by them when optical active lactic acids were previously added, was again found by another *dl*-former: with *Lactobacillus pentoaceticus*, inactive fermentation lactic acid was always obtained even in the presence of *d*- or *l*-lactic acid.

The form of fermentation lactic acids produced by *Strep. lactis bulgaricus* was found to be almost identical [*dl* + *d* (48%)], not only in the presence of *dl*-lactic acid as was mentioned above, but also *d*- or *l*-lactic acid. Therefore the bacteria was suggested to reveal the limited racemisation of the both forms of lactic acid.

From the manner of bacteria towards the lactic acids previously added to the fermentation liquids, lactic acid bacteria were classified into two types; the above mentioned *l*-former and *d*-formers were found to be the same type of bacteria with which any modification of the form of lactic acid produced by them was never observed, and very remarkable modification was pointed out with the other type of bacteria, including *dl*-formers and *dl* + *d*-former (*Strep. lactis bulgaricus*), as was already discussed.